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Oral Glucose Loading Acutely Attenuates Endothelium-Dependent Vasodilation in Healthy Adults Without Diabetes: An Effect Prevented by Vitamins C and E

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OBJECTIVES The goal of this study was to determine whether postprandial hyperglycemia, induced by oral glucose loading, attenuates endothelial function in healthy subjects without diabetes and whether coadministration of vitamins C and E could prevent these postprandial changes.

BACKGROUND Epidemiologic evidence suggests that postprandial hyperglycemia, below diabetic levels, is a risk factor for cardiovascular disease. Postprandial hyperglycemia may promote atherosclerosis through endothelial dysfunction and oxidative stress.

METHODS We evaluated the acute effects of oral glucose loading (75 g), alone and with vitamins C (2 g) and E (800 IU), on endothelium-dependent flow-mediated dilation (FMD) of the brachial artery, in a randomized, double-blind, placebo-controlled, crossover study of 10 healthy volunteers. Changes in the levels of markers of oxidative stress (plasma malondialdehyde and erythrocyte glutathione, glutathione peroxidase and superoxide dismutase) were also assessed.

RESULTS Increases in plasma glucose and insulin after glucose loading were unaffected by vitamin coadministration. With glucose loading alone, FMD fell from 6.5 ± 2.2 at baseline to 5.4 ± 1.7, 3.7 ± 2.1*, 4.1 ± 3.5* and 5.7 ± 1.9% at 1, 2, 3 and 4 h (*$p < 0.05$ vs. 0 h). In contrast, FMD did not change significantly after glucose plus vitamins (6.4 ± 1.3, 7.6 ± 1.8, 7.9 ± 2.7, 6.9 ± 2.3, 6.9 ± 1.9% at 0, 1, 2, 3 and 4 h). By two-way repeated measures analysis of variance we found a significant interaction between vitamin treatment and time ($p = 0.0003$), indicating that vitamins prevented the glucose-induced attenuation of FMD. Oxidative stress markers did not significantly change with glucose loading alone or with vitamins.

CONCLUSIONS Oral glucose loading causes an acute, transient decrease of FMD in healthy subjects without diabetes, which is prevented by vitamins C and E. (J Am Coll Cardiol 2000;36:2185–91) © 2000 by the American College of Cardiology

Diabetes mellitus is a well-established risk factor for cardiovascular disease (1). Increasingly, it has been suggested that postprandial hyperglycemia, at levels well below the cutoff for diabetes or impaired glucose tolerance (IGT), is also a risk factor for the development of cardiovascular disease (2–5). For example, several prospective epidemiologic studies of populations without diabetes, such as the Whitehall Study (6), Honolulu Heart Study (7) and Helsinki Policeman Study (8) have demonstrated that postprandial plasma glucose levels as low as 5.4 mmol/L are associated with an increased risk of eventual cardiovascular mortality. In fact, a meta-analysis of 20 published studies of nondiabetic cohorts indicates that there is a continuous and graded relationship between increasing fasting or postprandial glucose levels and the risk of cardiovascular events, with no apparent threshold (5).

The mechanisms by which postprandial hyperglycemia may promote atherosclerosis remain unknown. Evidence from in vitro studies suggests that hyperglycemia leads to increased generation of oxygen free radicals, such as superoxide anion, which may cause endothelial cell injury and inactivation of endothelial-derived nitric oxide (NO) (9–12). As a result, hyperglycemia causes endothelial cell dysfunction, which is a key step in the development of atherosclerosis (13). In humans, there is evidence that both type I and II diabetes mellitus are associated with impaired endothelium-dependent vasodilation (14,15) and increased oxidative stress (16,17). Even transient increases in plasma glucose may cause an acute impairment in endothelial function in healthy subjects without diabetes (18–20). As well, postprandial hyperglycemia, induced by oral glucose loading, may cause an acute oxidant stress in healthy subjects with normal glucose tolerance (20–23). Thus, it is plausible that repeated spikes in postprandial hyperglycemia may stimulate atherogenesis through endothelial dysfunction and oxidative stress (4,24).

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Abbreviations and Acronyms

ANOVA = analysis of variance
FMD = flow-mediated dilation
IGT = impaired glucose tolerance
IRCM = Institut de recherches cliniques de Montréal
NO = nitric oxide
SOD = superoxide dismutase

In vitro studies suggest that various antioxidants, including vitamins C and E, may prevent hyperglycemia-induced endothelial injury or dysfunction (9,12,25,26). In humans, acute administration of vitamin C improves endothelium-dependent vasodilation for patients with type II diabetes (27). Therefore, there may be a rationale for the use of vitamins C and E to reverse the vascular effects of hyperglycemia.

Accordingly, the purpose of this study was to confirm whether postprandial hyperglycemia, induced by an oral glucose load, attenuates endothelium-dependent flow-mediated dilation (FMD) and causes increased oxidative stress in healthy subjects without diabetes and to determine the time course of any such effects. Secondly, we examined whether coadministration of vitamins C and E could prevent these postprandial changes in endothelial function.

METHODS

Subjects. Ten healthy nondiabetic volunteers (6 men, 4 women), aged 20 to 30 years, were recruited from the hospital staff and associated university. All individuals were nonsmokers, normotensive and had total cholesterol < 6.2 mmol/L. None of the subjects had clinical evidence or a family history of premature cardiovascular disease. None of the subjects were taking medications or vitamin supplements. All subjects gave informed consent before participation. The study protocol was approved by the Research Ethics Committee of the Queen Elizabeth II Health Sciences Center.

Study design. The acute effects of oral glucose loading, with and without vitamins C and E, on FMD was studied in a randomized, double-blind, placebo-controlled, crossover design. All subjects were studied on two mornings, separated by 1 week. Individuals were randomized to receive 1 of 2 loads during the first study: 1) an oral glucose load (75 g glucose; Glucodex, Rougier, Chambly, Quebec, Canada) immediately followed by oral ingestion of placebo vitamins or 2) the same oral glucose load immediately followed by oral ingestion of vitamin C (2 g) and vitamin E (800 IU d-alpha-tocopheryl acetate). Subjects received the alternative load during the second study on the following week. The vitamins were administered in a double-blind fashion, with matching placebo capsules. The doses of vitamins C and E were selected because similar doses had been shown to reverse postprandial endothelial dysfunction after high fat or methionine loading (28,29).

Both studies were performed after a 12 h overnight fast. Caffeine and alcohol were avoided for 12 h before each study. During each study an indwelling venous catheter was inserted into the left antecubital vein for repeated venous blood samples. After at least 10 min rest, baseline blood samples for plasma glucose, insulin, serum lipids and markers of oxidative stress (including plasma malondialdehyde as a marker of lipid peroxidation and erythrocyte levels of the antioxidants: glutathione, glutathione peroxidase and superoxide dismutase [SOD]) were collected, and FMD measurements were performed at "time 0". Subjects then received their randomly assigned load, and serial blood samples and FMD measurements were obtained at 1, 2, 3 and 4 h after glucose loading.

Noninvasive endothelium-dependent FMD assessment. Endothelium-dependent FMD of the brachial artery was assessed noninvasively using a high-resolution ultrasound system (Hewlett-Packard SONOS 2500, Agilent Technologies, Palo Alto, California) with a 7.5 MHz linear-array vascular transducer, as previously described (30,31). Briefly, a pneumatic tourniquet placed proximally on the forearm was inflated to 250 mm Hg pressure for 5 min and rapidly deflated, resulting in reactive hyperemia. Brachial artery images were obtained at baseline and 1 min after cuff deflation to assess the change in brachial artery diameter in response to reactive hyperemia. This procedure was repeated at 1, 2, 3 and 4 h after glucose ingestion.

End-diastolic frames (coincident with the electrocardiographic R wave) from four consecutive cardiac cycles of the serial baseline and 1 min after reactive hyperemia phases (at time 0, 1, 2, 3 and 4 h) were digitized and analyzed by one observer, blinded to the load assignment and the protocol phase, as previously described (31). Endothelium-dependent FMD was calculated as the percent change in brachial artery diameter 1 min after reactive hyperemia compared with baseline at each time phase of the protocol (time 0, 1, 2, 3 and 4 h, within subject coefficient of variation—2.1%). Brachial artery flow and reactive hyperemia were estimated using velocity time integrals and brachial artery diameters, as previously described (30,31).

Biochemistry analyses. Venous blood was collected in EDTA tubes (for plasma glucose, insulin, malondialdehyde and erythrocyte glutathione, glutathione peroxidase and SOD) and in serum separating gel tubes (for serum lipids) and immediately placed on ice. Plasma was separated within 15 min and frozen at −70°C for subsequent analysis. After removal of plasma, erythrocyte samples were obtained by washing the remaining cells and then by repeated centrifugation to remove the liquid and buffy coat. The remaining packed erythrocytes were diluted with 9 parts ice-cold distilled water. These diluted (10%) erythrocyte aliquots were frozen at −70°C for subsequent analysis for glutathione, glutathione peroxidase and SOD levels.

Plasma glucose and serum lipids were measured with a Beckman Synchron CX7 system. Plasma insulin levels were measured with a commercial radioimmunoassay kit (Phad-

Table 1. Baseline Clinical Characteristics of the Study Subjects

Characteristic	n = 10
Age (yr)	25.5 ± 3.1
Men/Women	6/4
Body mass index (kg/m^2)	24 ± 3
Systolic BP (mm Hg)	118 ± 8
Diastolic BP (mm Hg)	72 ± 7
Heart rate (min^{-1})	63 ± 8
Total cholesterol (mmol/L)	5.1 ± 1.1
HDL-cholesterol (mmol/L)	1.3 ± 0.1
LDL-cholesterol (mmol/L)	3.3 ± 0.9
Triglycerides (mmol/L)	1.2 ± 0.5
Fasting glucose (mmol/L)	5.3 ± 0.7
Basal brachial artery diameter (mm)	3.59 ± 0.58
Baseline FMD (%)	7.0 ± 2.2

BP = blood pressure; FMD = flow-mediated dilatation; HDL = high-density lipoprotein; LDL = low-density lipoprotein.

eseph Insulin RIA, Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden). Plasma malondialdehyde was measured by high-performance liquid chromatography, as described by Fukunaga et al. (32). Erythrocyte glutathione was measured at Institut de recherches cliniques de Montréal (IRCM) by high-performance liquid chromatography and fluorescence detection, as described by Durand et al. (33), and results are expressed as μM/g of hemoglobin. Erythrocyte glutathione peroxidase and SOD levels were measured at IRCM using commercial kits (RANSEL and RANSOD, Randox Laboratories, Mississauga, Ontario), and results are expressed as U/g of hemoglobin. Serial erythrocyte glutathione peroxidase and SOD levels were measured in duplicate, and the results were averaged.

Statistical analysis. The effects of oral glucose loading, with and without vitamins, on plasma glucose, insulin, markers of oxidative stress and FMD were analyzed by repeated measures, one-way analysis of variance (ANOVA). Changes in these parameters over time were compared for glucose alone versus glucose plus vitamins by repeated measures, two-way ANOVA, with each patient acting as his own control in this crossover study. If differences reached statistical significance, post hoc analyses with a two-tailed paired *t* test was used to assess differences at individual time periods in the study, using a Bonferroni correction for multiple comparisons. Linear regression was used to assess the relationship between the change in FMD and the postprandial change in plasma glucose and insulin levels. Two-sided p values less than 0.05 were considered to indicate statistical significance. Continuous data are expressed as means ± standard deviation, unless stated otherwise.

RESULTS

The baseline characteristics of the 10 study subjects are shown in Table 1. Baseline blood pressure, fasting serum lipids, fasting glucose and FMD (forearm occlusion) were normal and were similar on both studies.

The effects of glucose loading, alone and with vitamins, on plasma glucose, insulin and serum lipids are shown in Table 2. Plasma glucose and insulin levels increased at 1 h postprandial and returned to baseline levels by 4 h with oral glucose loading alone. Of note, all subjects had a 2 h postprandial glucose level below the cutoff for IGT (7.8 mmol/L). Coadministration of vitamins C and E did not significantly affect the increase in plasma glucose and insulin levels after glucose loading. Serum lipids were unaltered after glucose loading, alone and with vitamins.

As seen in Table 3, heart rate, blood pressure, basal brachial artery diameter, basal brachial artery flow and reactive hyperemia did not change significantly after glucose loading, with or without vitamins.

The effects of glucose loading, alone and with vitamins,

Table 2. Glucose, Insulin and Lipid Values Before and After Oral Glucose Loading, Alone or With Vitamins

Variable	0 h	1 h	2 h	3 h	4 h	p Value
Plasma glucose–mmol/L						
Glucose	5.2 ± 0.7	7.9 ± 3.0	5.8 ± 1.9	4.5 ± 1.3	4.6 ± 0.5	
Glucose + vitamins	5.5 ± 0.4	8.3 ± 2.4	6.2 ± 1.4	4.7 ± 0.6	5.0 ± 0.5	0.99
Plasma insulin—mIU/L						
Glucose	6.9 ± 2.5	69.8 ± 44.2	38.2 ± 26.5	13.4 ± 14.5	6.6 ± 2.3	
Glucose + vitamins	5.9 ± 1.1	53.6 ± 27.2	30.2 ± 14.6	9.2 ± 4.3	6.7 ± 2.5	0.35
Total cholesterol—mmol/L						
Glucose	5.1 ± 1.0	4.9 ± 0.9	4.8 ± 0.9	5.0 ± 0.9	5.0 ± 0.9	
Glucose + vitamins	5.0 ± 0.7	4.8 ± 0.6	4.7 ± 0.6	4.8 ± 0.6	4.8 ± 0.7	0.74
LDL-cholesterol—mmol/L						
Glucose	3.3 ± 0.9	3.2 ± 0.8	3.2 ± 0.9	3.3 ± 0.9	3.4 ± 0.9	
Glucose + vitamins	3.2 ± 0.5	3.0 ± 0.5	3.0 ± 0.5	3.1 ± 0.5	3.1 ± 0.6	0.14
HDL-cholesterol—mmol/L						
Glucose	1.3 ± 0.1	1.2 ± 0.2	1.2 ± 0.2	1.3 ± 0.2	1.3 ± 0.2	
Glucose + vitamins	1.3 ± 0.1	1.3 ± 0.2	1.2 ± 0.2	1.3 ± 0.2	1.2 ± 0.2	0.11
Triglycerides—mmol/L						
Glucose	1.2 ± 0.5	1.1 ± 0.4	0.9 ± 0.4	0.9 ± 0.3	0.8 ± 0.3	
Glucose + vitamins	1.1 ± 0.5	1.1 ± 0.5	1.0 ± 0.4	0.9 ± 0.5	0.9 ± 0.5	0.10

HDL = high-density lipoprotein; LDL = low-density lipoprotein.

Table 3. Hemodynamics, Basal Brachial Artery Diameters, Brachial Artery Flow and Reactive Hyperemia Before and After Oral Glucose Loading, Alone or With Vitamins

Basal Hemodynamics	0 h	1 h	2 h	3 h	4 h	p Value
Systolic blood pressure (mm Hg)						
Glucose	117 ± 8	116 ± 8	114 ± 8	112 ± 8	114 ± 11	
Glucose + vitamins	116 ± 10	120 ± 9	113 ± 10	113 ± 10	116 ± 9	0.32
Diastolic blood pressure (mm Hg)						
Glucose	71 ± 7	69 ± 9	70 ± 7	67 ± 7	71 ± 10	
Glucose + vitamins	73 ± 8	70 ± 10	69 ± 10	71 ± 10	71 ± 10	0.29
Heart rate (min^{-1})						
Glucose	63 ± 9	64 ± 8	63 ± 9	60 ± 9	64 ± 9	
Glucose + vitamins	62 ± 8	62 ± 8	59 ± 8	59 ± 7	58 ± 6	0.11
Basal brachial artery diameter (mm)						
Glucose	3.58 ± 0.63	3.62 ± 0.61	3.64 ± 0.67	3.62 ± 0.62	3.60 ± 0.59	
Glucose + vitamins	3.61 ± 0.58	3.66 ± 0.57	3.65 ± 0.53	3.64 ± 0.57	3.58 ± 0.54	0.63
Basal brachial artery flow (ml/min)						
Glucose	134 ± 105	108 ± 116	85 ± 45	106 ± 96	89 ± 58	
Glucose + vitamins	100 ± 69	107 ± 89	90 ± 67	88 ± 75	76 ± 41	0.10
Reactive hyperemia (%)						
Glucose	421 ± 195	614 ± 240	595 ± 83	544 ± 275	596 ± 149	
Glucose + vitamins	582 ± 180	576 ± 290	646 ± 201	656 ± 193	729 ± 327	0.65

on FMD are shown in Figure 1. Flow-mediated dilation significantly fell from 6.5 ± 2.2% at baseline to 3.7 ± 2.1% at 2 h postprandial and returned towards baseline by 4 h with glucose loading alone ($p = 0.002$ by one-way ANOVA). In contrast, FMD did not change significantly in response to glucose plus vitamins ($p = 0.20$ by one-way ANOVA). Using two-way repeated measures ANOVA (treatment group vs. time), we found a significant treatment group effect ($p = 0.0016$) but no significant time effect ($p = 0.28$). There was a significant interaction between treatment group and time ($p = 0.0003$), indicating that there was a significant difference between glucose alone versus glucose with vitamins with regard to the change in FMD. Thus, vitamins C and E prevented the acute attenuation of FMD induced by glucose loading. Accordingly, the mean change in FMD seen at 2 h postprandial compared with preprandial endothelial function was significantly different for glucose alone versus glucose with vitamins (Fig. 2). The change in FMD induced by glucose loading did not correlate to the postprandial change in plasma glucose or insulin levels.

As shown in Table 4, the systemic markers of oxidative stress did not change significantly with glucose loading alone or with the coadministration of vitamins C and E.

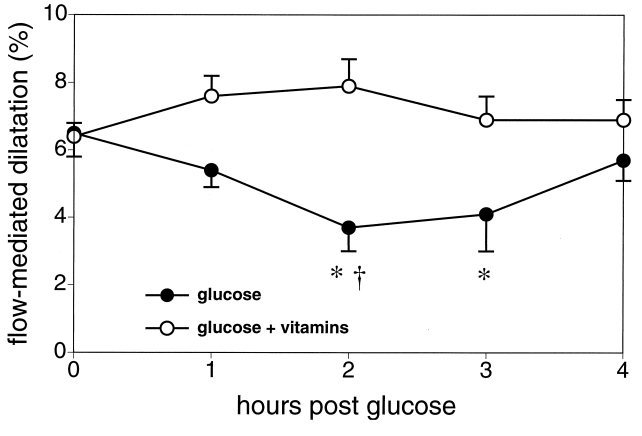

Figure 1. Effects of oral glucose loading alone, **(solid circle)**, or with vitamins C and E, **(open circle)** on flow-mediated dilatation (FMD; mean ± SE) over 4 h. Vitamins C and E prevented the acute attenuation of FMD seen with glucose loading alone ($p = 0.0003$ by two-way analysis of variance for response of glucose alone versus glucose with vitamins). *$p < 0.05$ compared with FMD at baseline (0 h); †$p < 0.05$ compared with glucose with vitamins at 2 h.

DISCUSSION

The major findings of this study are: 1) oral glucose loading causes an acute, transient attenuation of endothelium-dependent FMD in healthy subjects without diabetes and 2)

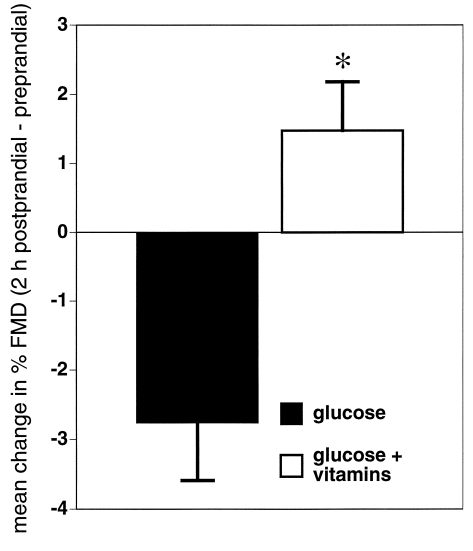

Figure 2. Mean change in flow-mediated dilatation (FMD) at 2 h postprandial compared with preprandial FMD for glucose loading alone **(solid bar)** versus glucose loading plus vitamins **(open bar)**. The bars represent mean ± standard error. *$p = 0.001$ for glucose alone versus glucose with vitamins.

Table 4. Systemic Markers of Oxidative Stress Before and After Oral Glucose Loading, Alone or With Vitamins

Variable	0 h	1 h	2 h	3 h	4 h	p Value
Plasma malondialdehyde (nmol/ml)						
Glucose	0.98 ± 0.29	1.06 ± 0.43	0.89 ± 0.26	0.94 ± 0.30	1.02 ± 0.21	
Glucose + vitamins	1.06 ± 0.34	1.00 ± 0.34	0.92 ± 0.31	0.92 ± 0.30	0.93 ± 0.28	0.15
Erythrocyte glutathione (μM/g Hb)						
Glucose	3.1 ± 0.6	3.2 ± 0.6	3.0 ± 0.5	3.1 ± 0.7	3.1 ± 0.6	
Glucose + vitamins	3.0 ± 0.7	3.1 ± 0.8	3.1 ± 0.6	3.2 ± 0.8	3.0 ± 0.8	0.34
Erythrocyte glutathione peroxidase (U/g Hb)						
Glucose	42.2 ± 22.7	39.5 ± 19.1	41.7 ± 20.7	43.3 ± 18.7	44.9 ± 17.1	
Glucose + vitamins	42.3 ± 20.9	39.2 ± 18.5	36.6 ± 17.9	41.6 ± 21.2	37.6 ± 15.7	0.25
Erythrocyte SOD (U/g Hb)						
Glucose	728 ± 98	670 ± 69	708 ± 100	681 ± 96	735 ± 155	
Glucose + vitamins	679 ± 79	691 ± 157	696 ± 93	682 ± 107	679 ± 122	0.37

Hb = hemoglobin; SOD = superoxide dismutase.

coadministration of vitamins C and E prevented this postprandial change in endothelial function.

Effect of glucose loading on endothelial function. It is well established that diabetes is associated with impaired endothelium-dependent vasodilation (14,15). However, even acute hyperglycemia may attenuate endothelium-dependent vasodilation in subjects without diabetes (18–20). Williams et al. (18) first demonstrated that 6 h of local hyperglycemia (16.7 mmol/L) induced by glucose clamping impaired endothelium-dependent vasodilation in humans without diabetes. Similar findings were found when octreotide was coadministered to block the release of insulin, suggesting that these vascular effects were mediated by hyperglycemia, rather than hyperinsulinemia. Recently, it has been demonstrated that mild increases in postprandial glucose levels may alter endothelial function. For example, Akbari et al. (19) demonstrated that ingestion of 75 g of glucose by healthy subjects without diabetes could decrease FMD after 1 h. Similarly, Kawano et al. (20) demonstrated that FMD decreased within 1 h of oral glucose loading in subjects with normal glucose tolerance, IGT and diabetes. However, the degree of postprandial impairment was greater with IGT and diabetes than it was with normal glucose tolerance. In that study there was a negative correlation between FMD and plasma glucose, suggesting that there is a continuous and graded relationship between increasing glucose levels and decreasing endothelial function. Our results confirm these observations because FMD was significantly attenuated 2 h after oral glucose loading and returned towards baseline by 4 h. Similar to the previous studies, this change in endothelial function occurred at relatively low levels of postprandial hyperglycemia, which was below the cutoff for IGT and diabetes. Unlike Kawano et al. (20) we did not find any relationship between endothelial function and plasma glucose although our study was confined to subjects with normal glucose tolerance and had fewer subjects.

Potential mechanisms for endothelial dysfunction after glucose loading. Flow-mediated dilation of the brachial artery has been shown to be dependent upon the release of endothelium-derived NO (34). Therefore, our findings imply that the oral glucose loading may lead to a transient loss of NO bioavailability, which may be a consequence of either decreased NO formation or increased NO inactivation. In vitro studies suggest that the exposure of endothelial cells to elevated glucose levels leads to the generation of oxygen free radicals, such as superoxide anion, which may inactivate NO or contribute to endothelial cell injury (9–12).

In humans, there is evidence that hyperglycemia is associated with increased oxidative stress. Patients with diabetes mellitus have increased levels of lipid peroxidation and reduced levels of endogenous antioxidants compared with controls (16,17). Moreover, acute oral glucose loading can cause an acute oxidant stress in healthy, subjects without diabetes (20–23). In contrast, our study failed to demonstrate any significant changes in a number of systemic markers of oxidative stress, including malondialdehyde, erythrocyte SOD, glutathione and glutathione peroxidase after glucose loading. The absence of an effect on these systemic markers may suggest that our study was not able to detect small differences in our limited sample size or that these markers do not reflect minute changes occurring within the vascular wall.

Potential mechanisms for the improvement in postprandial endothelial function with vitamins C and E. There are many theoretical mechanisms whereby vitamins C and E may have prevented postprandial changes in endothelial function in our study. Importantly, our study design does not allow us to differentiate the effects of vitamin C over vitamin E although, theoretically, their effects may be synergistic (35). It has been suggested that "antioxidant" vitamins may improve endothelial function by quenching superoxide anion, thereby preventing NO inactivation (27). However, this is unlikely, as the rate constants for the interaction between vitamins C and E and superoxide are significantly lower than those for the reaction between superoxide anion and NO (36). Alternatively, vitamins C and E may prevent low-density lipoprotein oxidation (37). Oxidized low-density lipoprotein can directly inactivate NO

and cause endothelial injury (37). Lastly, vitamins C and E may directly inhibit hyperglycemia-mediated superoxide anion release from the vascular wall (12,25), thus preventing the inactivation of NO.

Study limitations. First, as oral glucose loading produced significant increases in both plasma glucose and insulin levels, we cannot conclude that the change in FMD was a result of elevations of plasma glucose rather than insulin. However, in vitro studies demonstrate that acute hyperglycemia itself may cause impaired endothelium-dependent vasodilation (9,10). Moreover, Williams et al. (18) demonstrated that the effects of acute hyperglycemia on endothelium-dependent vasodilation were similar after blocking insulin release with octreotide, suggesting that hyperglycemia is likely the culprit. Second, the possibility that glucose loading caused a nonspecific loss of smooth muscle cell function cannot be excluded because we did not specifically measure endothelium-independent nitroglycerin-mediated dilation in our study. However, previous studies have shown that oral glucose loading had no effect on nitroglycerin-mediated dilation (19,20), suggesting that our observed effects are more likely to be endothelium-dependent. Although the study had a limited number of subjects, measurement of FMD has been shown to be reproducible with minimal variability over time, which allows us to use this technique to detect significant differences in small groups by using each subject as their own control (38,39). While our baseline FMD (7%) may seem lower than the "normal" values reported in some previous studies (30), our values are consistent with other studies of young healthy subjects using a cuff position below the elbow (40–42). In premenopausal women, FMD may vary during the menstrual cycle (43). Although we did not control for the menstrual cycle phase in female subjects, there was little variation in their baseline (fasting) FMD measurements on the two study days.

Clinical implications and conclusions. Our results demonstrate that oral glucose loading transiently attenuates endothelium-dependent vasodilation in healthy humans without diabetes, which can be prevented by the coadministration of vitamins C and E. The clinical relevance of these findings may be emphasized by epidemiologic studies that have shown that postprandial hyperglycemia at levels well below the cutoff for diabetes or IGT is a risk factor for cardiovascular disease. As endothelial dysfunction is a key event in atherogenesis, our findings suggest that there may be a role for vitamin C and E supplementation in preventing daily postprandial insults to endothelial function, which may eventually contribute to atherosclerotic vascular disease. However, further studies assessing the long-term effects of vitamin supplementation on postprandial endothelial function and cardiovascular disease are required.

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